REGULAR OCCURRENCE OF HETEROPLOIDY IN A GROUP OF PENTATOMIDAE (HEMIPTERA)

FRANZ SCHRADER

Department of Zoology, Columbia University, New York

The testis of all pentatomid Hemiptera is composed of lobes which are constant in number for any given species. In some species the size of the spermatocyte cells and sperms may vary in the different lobes, but in any one species there is a characteristic size for each lobe. In other words, if the first lobe in one specimen produces unusually large spermatocytes, the corresponding lobe in other specimens of that species will also show such large spermatocytes. This peculiarity was first discovered by Montgomery (1898), who described it in some detail in a later paper (1910). The phenomenon was called "polymegaly" by Bowen (1920, 1922a, 1922b) who in his exact analysis laid special stress on the processes of sperm formation. He found that certain cytoplasmic elements varied in amount proportionally to the size of the cells, but that the size of the chromosomes varied little or not at all in the different lobes. Normal sperms were formed from all lobes, and differences in the shape of the head arose only because there was greater elongation in some lobes.

The conditions that are basic to this constant variation are therefore subject to thoroughly regulated processes in any given species. They involve no mitotic irregularities and the occurrence of nondisjunction, multipolar spindles and failure of cytoplasmic division, which of course are found here as in other animals and plants, always seem to result from physiological accidents that are sporadic and in no way correlated with polymegaly.

It is therefore of some interest to record a series of cases among the Pentatomidae in which mitotic disturbances occur regularly in a certain lobe of the testis and affect all the cells of that lobe. Moreover such irregularity, although resulting in heteroploidy of an extremely large range, does not affect the basic processes of sperm formation. Hence spermatozoa are produced which vary greatly in volume, but which appear to be otherwise normal in general structure.

Material and Methods

The pentatomids concerned are *Loxa flavicollis* Drury, of which nine specimens were collected in Panama (1940 and 1941) and Costa Rica (1944); *Loxa picticornis* Horvath, represented by six specimens from Panama (1940) and Costa Rica (1944); and *Loxa florida* Van Duz, collected in Florida, U. S. A., in 1909. The data on *Loxa florida* are contained in Bowen's papers (1922a and b) although the main points here at issue are not specifically noted by him. A fourth species, *Mayrinia variegata* Dist., is represented in my material by only a single male collected in Costa Rica in 1944. Though strikingly smaller than the uniformly large species of Loxa, it is nevertheless a close relative and was formerly classified in that genus. Its affinity to the species of Loxa is now further attested by the fact that it shares with them the striking mitotic features that form the subject of this paper.

Both testes and ovaries were fixed in either Sanfelice or Allen's Bouin and stained with haematoxylin, gentian violet, or the Feulgen method. Although Bowen did not state it, it is more than likely that his material of *Loxa florida* for which he thanks Professor E. B. Wilson, was fixed in strong Flemming solution. In all of my own material the gonads were usually fixed within a short interval of capture, usually less than three hours and sometimes within a few minutes.

My thanks are due to a number of people who have aided me in collecting my material. In this respect I am especially obligated to Dr. T. J. Grant of the U. S. Department of Agriculture in Turrialba, Costa Rica.

Structure of the Testis

In all three species of Loxa, the testis is composed of seven lobes, which are arranged side by side in a single series. Following Bowen (1922b) these lobes are designated by consecutive numbers, the first being closest to the side toward which the vas deferens opens (Fig. 1). The lobes show a constant variation in diameter, but only one lobe is strikingly larger than the others. This is the fifth, which may have a diameter two or three times that of any other and which at the same time is characterized by spermatogonial and early spermatocyte cells that are distinctly smaller than the cells of other lobes. It is this lobe which regularly shows heteroploidy in the spermatocytes.

It is this lobe also which shows heteroploidy in Mayrinia. There however, the fifth is not the largest lobe, for it is exceeded in diameter by the sixth. In neither of the two Mayrinia testes at my disposal is a seventh lobe visible, and it may be that in this species the last two lobes have fused to form a single, larger one. Since there are some indications that my specimen is an old male, it is inadvisable to draw any general conclusions on this point. The main point however is fully established. In all four species it is the fifth lobe of the testis which always shows the special development here under discussion.

Cytology

In all three species of Loxa, the spermatocyte cells are smaller in the fifth than in the other lobes. But as Bowen (1922b) has pointed out in reference to polymegaly in general, this size difference rests in the volume of the cytoplasm rather than that of the chromosomes. In Mayrinia, such polymegaly is not as striking as in Loxa.

The spermatogonial divisions appear to be very much alike in all of the lobes (Fig. 2*A* and *B*). The fifth lobe shows the diploid set of 14 chromosomes differing in no perceptible way from that of the remaining lobes, and mitoses are orthodox. The unusual developments do not become evident until the prophase of the first spermatocyte. Since a detailed cytological analysis will be published elsewhere, only a brief outline will be given here.

The most obvious deviation from the normal course first occurs some time before diakinesis. When the cells of a given cyst reach this stage they begin to

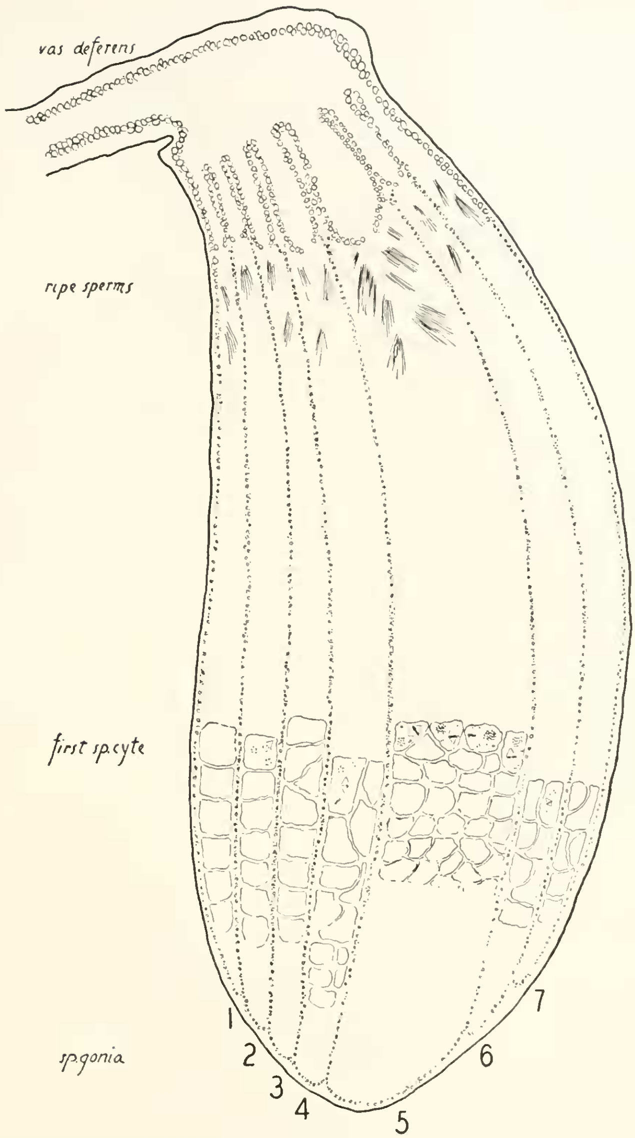

FIGURE 1. Section of the testis of *Loxa flavicollis*, showing the positions and comparative sizes of the seven lobes. Heteroploidy occurs regularly in the fifth lobe. Actual length of testis is 4 mm. and contents are only partially shown.

fuse with each other. The process is irregular in the sense that cellular aggregates resulting from the union of two or very few cells may lie side by side with much larger aggregates comprising many more cells. The nuclei at this time tend to become amoeboid and may divide by constriction, but if fusion occurs among them, it is not general.

Later during the prometaphase, cellular boundaries become very vague and the whole cyst may come to simulate a single, giant cell. It is at this time also that the nuclear walls disintegrate, as they do in the normal course of events. The chromosomes, condensing rapidly, then seem to lie scattered irregularly through the cytoplasm. However, immediately prior to the first appearance of spindles, the cytoplasm is segregated into discrete masses which do not seem

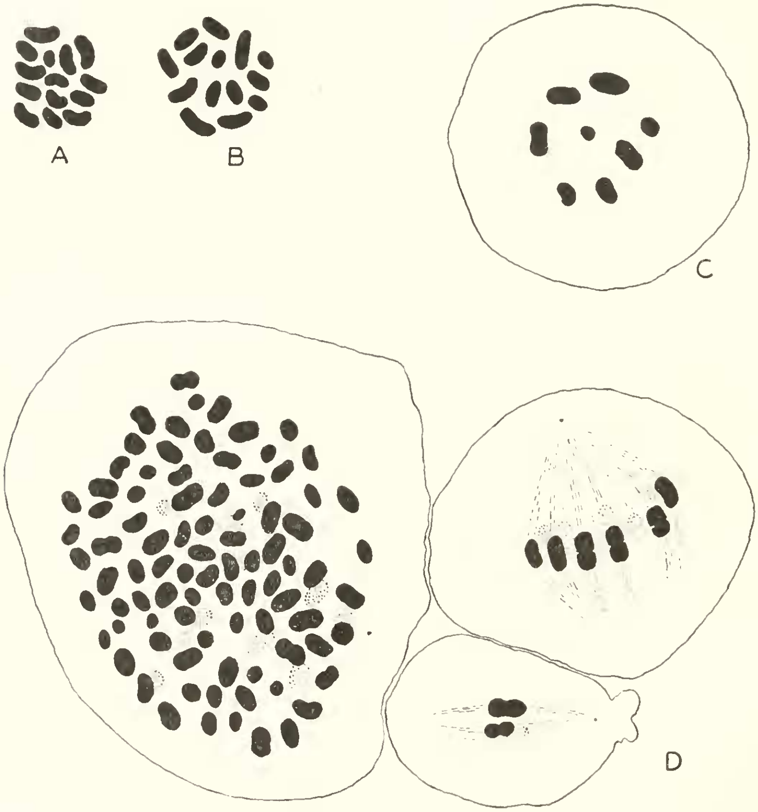

FIGURE 2. All from *Loxa flavicollis* (approx. 1,500 ×).
A. Spermatogonial metaphase from normal lobe (14 chromosomes).
B. Spermatogonial metaphase from fifth lobe (14 chromosomes).
C. First spermatocyte metaphase from normal lobe (6 autosomal tetrads + X + Y).
D. First spermatocyte metaphases from fifth lobe (showing 3, 12, and upwards of 60 chromosomal bodies).

to correspond exactly to the original fusion aggregates. It is probable that this new step involves the activity of both chromosomes and centrioles in the formation of spindles, and the latter appear very soon thereafter. Simultaneously the chromosomes are arranged into numerous metaphase plates, the number of which corresponds to the rounded masses of cytoplasm just formed. Since the latter seem to have no reference to the original prophase spermatocyte cells, the different metaphases may be composed of as few as two (perhaps even one) and as many as two hundred or more chromosomes (Fig. *2D*). By the same token, the types of chromosomes included in such metaphase plates vary greatly. All in all, these configurations of chromosomes are the result of rather haphazard processes and it is probably only very rarely that they happen to represent the orthodox set of chromosomes (Fig. *2C*) in the first spermatocyte (i.e. six autosomal tetrads + X + Y), especially since it seems doubtful whether normal pairing occurs in the fifth lobe.

Although the number of multipolar spindles is distinctly higher than in normal lobes, it is not as great as one might expect from the seemingly confused conditions that prevail just before spindle formation. Unlike the chromosomal movements in the preceding period, the maneuvers of the centrioles are evidently not completely

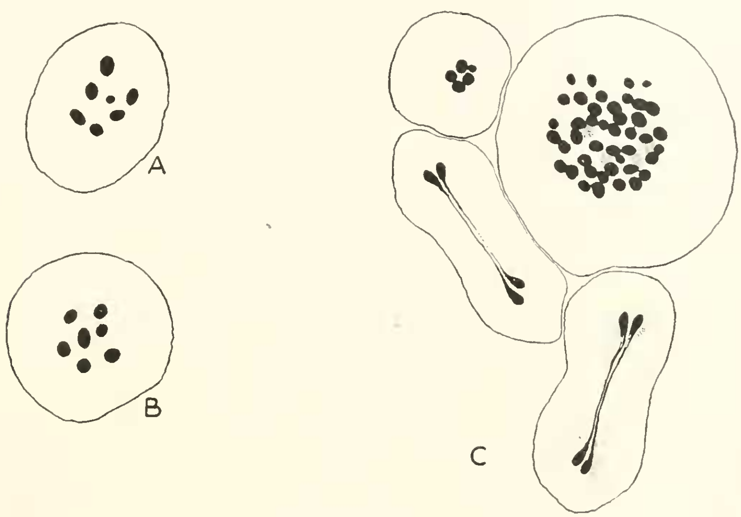

FIGURE 3. All from *Loxa flavicollis* (approx. 1,500 ×).
A. Chromosomes of telophase of second spermatocyte in normal lobe (6 autosomes + Y).
B. Chromosomes of telophase of second spermatocyte in normal lobe (6 autosomes + X).
C. Chromosomes of telophase of second spermatocyte in fifth lobe (showing 2, 4, 5, and upwards of 40 chromosomes at each pole).

at random and their final positions result in bipolar figures in the great majority of cases.

Despite the bewildering range in the composition of these metaphase plates, the actual division of the abnormal first spermatocytes occurs normally. The second spermatocytes therefore carry a correspondingly great variation in the numbers of chromosomes and they too divide successfully to give spermatid cells (Fig. 3).

The nuclei that are formed in the spermatids after the second spermatocyte division of course reflect the variation in the chromosome numbers that are encountered in the meiotic divisions. Hence they may greatly exceed in size the normal spermatid nucleus, and such large nuclei may lie side by side with the tiny nuclei derived from only one or two chromosomes (Fig. 4). However except for this deviation from the normal size, the processes of sperm formation seem to parallel those observed in the normal lobes. The behavior of chondriosomes and Golgi material seems to be regular, and the transformations of the nucleus that culminate in the tenuous head of the ripe sperm are undergone just as in normal cells. The number of degenerating sperms is not great and certainly a huge number reach the final stages near the entrance to the vas deferens. Since cells with a normal complement of chromosomes must be very rare indeed, there is no escaping the fact that very many sperms carrying irregular combinations and numbers of chromosomes reach the final stages of development. Whether they then enter the egg and become functional has not been determined.

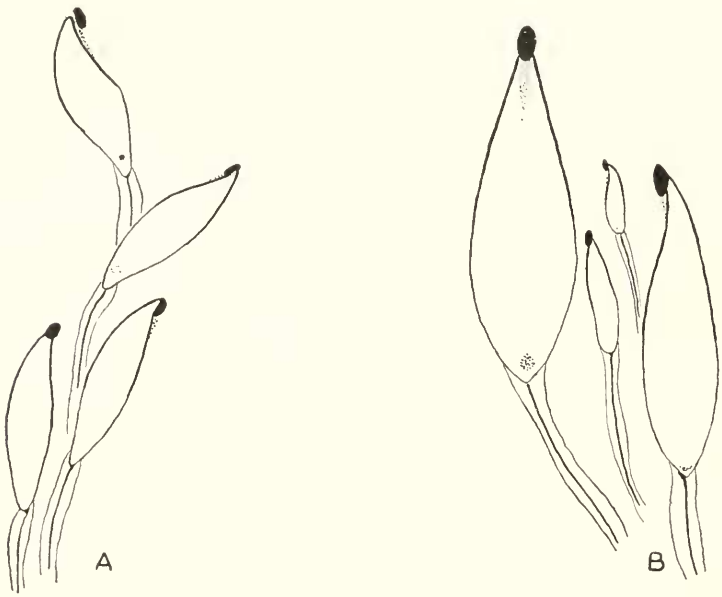

FIGURE 4. All from *Loxa flavicollis* (approx. 1,500 ×).
A. Sperms (only heads shown complete) at about Stage n. from normal lobe.
B. Sperms (only heads shown complete) at about Stage n. from fifth lobe, showing great variation in size.

The evidence that *Loxa florida* duplicates the conditions here described, is indirect but conclusive. In one of his papers, Bowen (1922a) discusses the occurrence of abnormally high numbers of chromosomes in that species, and ascribes them to a fusion of spermatocyte cells. He mentions that this takes place in the same lobe of both the Loxa testes available to him. Although not stating which lobe this is, Bowen characterizes it as carrying unusually small spermatogonial and early spermatocyte cells. In a somewhat later paper of that year (1922b) he mentions that in *Loxa florida* it is the fifth lobe that has cells which are distinctly smaller than those of the other lobes. There is thus no reason to doubt that he was dealing with conditions in *Loxa florida* that are essentially the same as those here described and which there occur also in the fifth lobe of the testis.

Evolutionary Aspects

These then are the astonishing conditions that confront us in the two genera, Loxa and Mayrinia. In four different species, taxonomically distinct, and collected from Panama, Costa Rica, and Florida—a latitudinal range of at least 1,700 km. (or about 1,050 miles)—one of the lobes of every testis undergoes a very special type of abnormal development. This abnormality results in a great number of unusual distributions of the chromosomes, but is in its basic nature identical in all four species. For in all of them it is always the fifth lobe of the testis that is affected; in all of them the spermatogonial divisions of this lobe seem normal and cytological indications of abnormality do not appear until the preparatory phases of the meiotic period; and in all four species the odd assortments of chromosomes that result partake nevertheless in a formation of sperms which are normal in appearance except for size.

Whatever the factors may be that bring about this constant and regularly occurring abnormality, its evolutionary aspects pose some interesting questions. It is reasonable to assume that the conditions underlying it are of long standing in the history of these species and that in fact they were probably present in the parent form from which they arose. Since all of my sixteen specimens carry the orthodox complement of 14 chromosomes, the indications are that the great majority of the sperms with abnormal chromosomes numbers are either nonfunctional or produce nonviable zygotes. The existence of so wasteful a development would seem to be an incongruity which might well be expected to meet with short shrift in the processes of natural selection. Nevertheless it has persisted, either because it involves some benefit to the species that is not immediately obvious or else because the elaboration of useless sperms, even in such huge numbers, entails only an imperceptible drain on the evolutionary welfare of the animal since it is amply compensated by the production of sperms from the normal lobes of the testis.

Be that as it may, the constant production of gametes with irregular chromosome numbers should greatly enhance the chances for producing zygotes with other than the usual complement of 14 chromosomes. Granted that most of the abnormal sperms will not produce viable zygotes, it is likely that some of the many unusual combinations do at times develop. Certainly the flow of new and heteroploid forms from such a species should be very much greater than in the case of species in which the opportunities for such varietal changes are restricted to the comparatively rare mitotic accidents that occur in the more orthodox processes of germ cell formation. It may be that a basic conservation that may exist among the Pentatomidae is not favorable to such evolutionary adventures, but the number of specimens at my disposal is surely too small to allow of any final conclusions on this point. Certain it is that a further examination of the 33 so far described species that belong to the three, taxonomically closely knit genera Loxa, Mayrinia, and Chlorocoris may well throw light on these questions (all except *Loxa florida* are exclusively neotropical, but their range extends from Florida to Argentina; see Van Duzee, 1909 and Horvath, 1925).

But if, as already suggested, the conservatism of these Hemiptera does not allow a utilization of such evolutionary opportunities, the case nevertheless presents a mechanism which in some other groups might well be conducive to rapid and great strides in the elaboration of new species. The ready viability of forms with unusual chromosome combinations such as characterizes for instance genera like Datura and Crepis would suggest that other forms might well take advantage of such a range of possibilities. At any rate, the case indicates in what unexpected ways a group of organisms may suddenly become very active in the evolutionary sense.

Differentiation of Sperms

The case reflects in a rather striking, albeit not entirely novel way, on the problem of differentiation. It is clear that the complex processes which bring about the formation of the highly specialized sperm—including the special transformations of Golgi and chondriosome material as well as the change of the spherical spermatid nucleus to the extremely tenuous, chromatic sperm head—all occur regardless of whether only one or two or many dozens of chromosomes are present in the cell. It therefore would seem safe to conclude that the immediate control over these developments is vested in cytoplasmic elements and that, if nuclear genes are primarily responsible they must exert their influence on the cytoplasm at least two mitotic cycles earlier (in the spermatocyte prophase when cell fusion first occurs). A demonstration to the same effect is contained in the work of Dobzhansky and Sturtevant (1931) where it is shown that sperms of Drosophila which have lost various sections of the chromosomes through translocation during the meiotic prophase, nevertheless become functional.

Summary

1. The fifth lobe in the testes of four species of Loxa and Mayrinia regularly produces sperms carrying heteroploid complements of chromosomes.

2. This condition must be of long evolutionary standing and may be of significance in the formation of new forms.

LITERATURE CITED

Bowen, R. H., 1920. Studies on insect spermatogenesis I. *Biol. Bull.*, **39**: 316–363.

Bowen, R. H., 1922a. Notes on the occurrence of abnormal mitoses in spermatogenesis. *Biol. Bull.*, **43**: 184–203.

Bowen, R. H., 1922b. Studies on insect spermatogenesis IV. *Proc. Am. Ac. Arts. Sci.*, **57**: 391–423.

Dobzhansky, T., and A. H. Sturtevant, 1931. Translocation between the second and third chromosomes of Drosophila and their bearing on Oenothera problems. *Carnegie Inst. Publ.*, **421**: 25–29.

Horvath, G., 1925. De pentatomidarum genere Loxa Am. Serv. et de novo genere ei affini. *Ann. Mus. Nat. Hungarici*, **22**: 307–328.

Montgomery, T. H., 1898. The spermatogenesis in Pentatoma up to the formation of the spermatid. *Zool. Jahrb., Anat.*, **12**: 1–88.

Montgomery, T. H., 1910. On the dimegalous sperm and chromosomal variation of Euschistus, with reference to chromosomal continuity. *Arch. Zellf.*, **5**: 120–146.

Van Duzee, E. B., 1909. Observations on some Hemiptera taken in Florida in the spring of 1908. *Bull. Buffalo Soc. Nat. Sci.*, **9**: 149–230.